Characterization of *Anaeromyxobacter dehalogenans* and Related Metal-Reducing Bacteria by MALDI-TOF MS
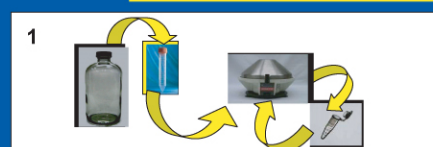
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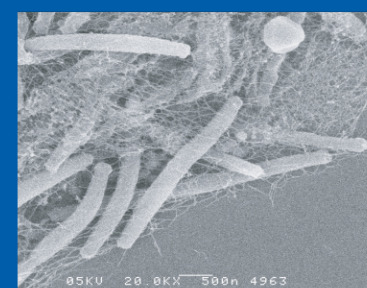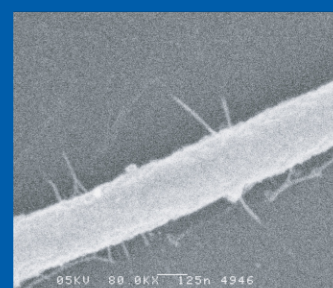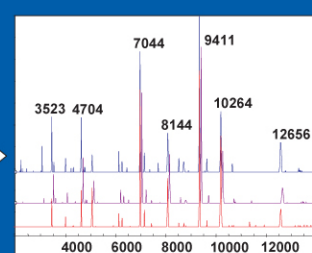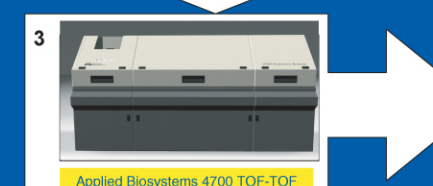
Procedure

1. Sample preparation:
   a. collect and centrifuge cells
   b. wash cell pellet twice with sucrose buffer
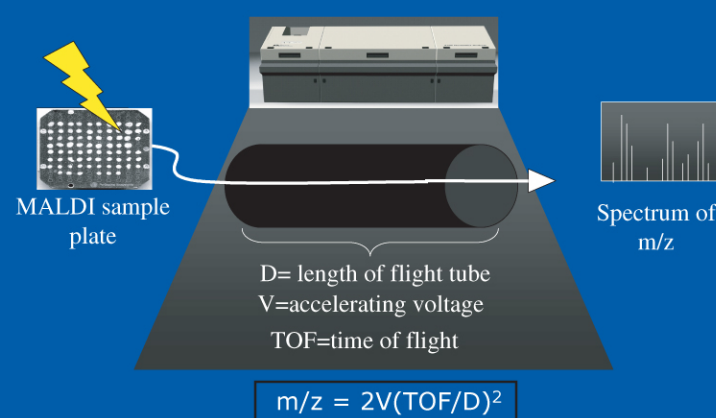
2. Spot sample on MALDI plate

3. Analyze by MALDI-TOF

MALDI-TOF Mass Spectrometry

MALDI sample plate

Spectrum of m/z

D= length of flight tube
V=accelerating voltage
TOF=time of flight

$$m/z = 2V(TOF/D)^2$$

Organisms Analyzed
(with abbreviations)

Organism	Electron Donor	Electron Acceptor
Anaeromyxobacter dehalogenans str. 2CP-C 2CP-C (Fumarate)	Acetate	Fumarate
Anaeromyxobacter dehalogenans str. 2CP-C 2CP-C (Nitrate)	Acetate	Nitrate
Anaeromyxobacter dehalogenans str. 2CP-C 2CP-C (2-CP)	Acetate	2-Chlorophenol
Anaeromyxobacter dehalogenans str. 2CP-1 2CP-1	Acetate	Fumarate
Anaeromyxobacter dehalogenans str. 2CP-3 2CP-3	Acetate	Fumarate
Anaeromyxobacter dehalogenans str. R R	Acetate	Fumarate
Desulfuromonas michiganans str. BB1 DS str. BB1	Acetate	Fumarate
Geobacter sp. str. SZ Geo str. SZ	Acetate	Fumarate

Phylogeny: The metal reducers analyzed are closely genetically related.

ANAEROMYXOBACTER

Spectra of Metal-Reducing Bacteria and *E. Coli*

Genus Specificity: Typical spectra of *Geobacter*, *Desulfuromonas*, and *Anaeromyxobacter*. These fingerprints are easily, visually distinguished. *E. Coli* is included as a Gram-negative control.

Gel-view Representation: Spectra of 2CP-C Grown with Various Electron Acceptors

Growth Conditions: This gel-view translation facilitates visualization of the minor differences between mass spectra of 2CP-C grown with different substrates.

Classification

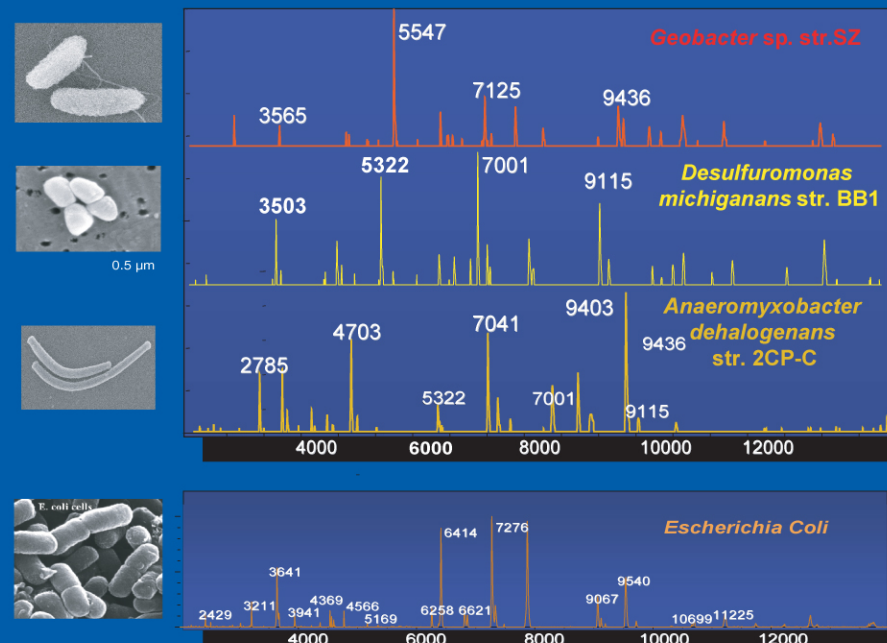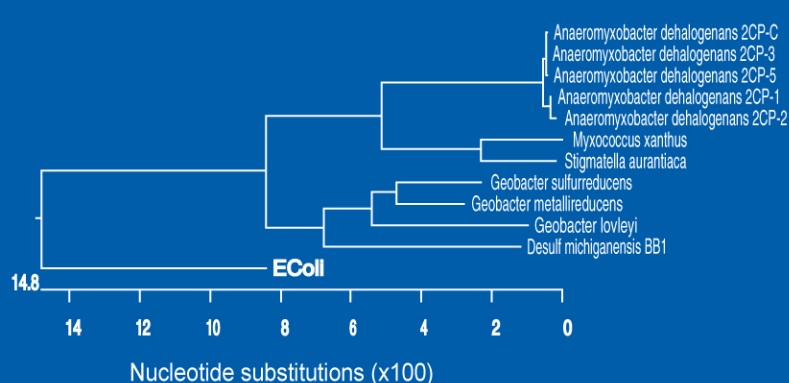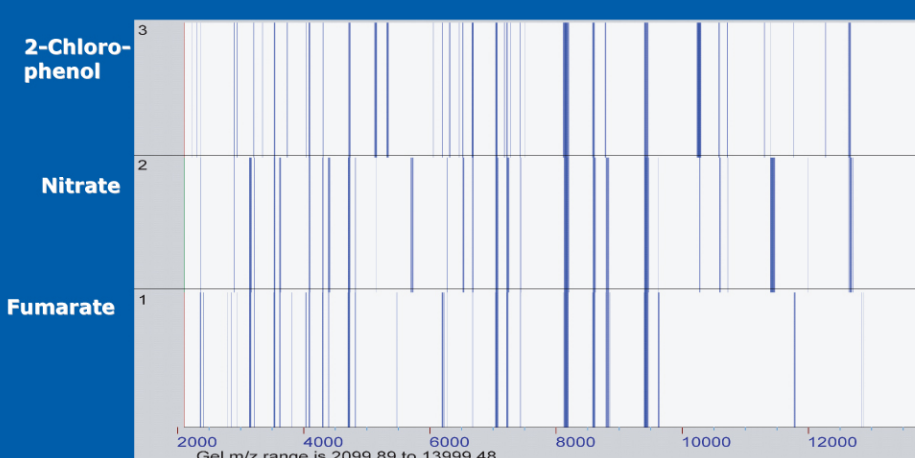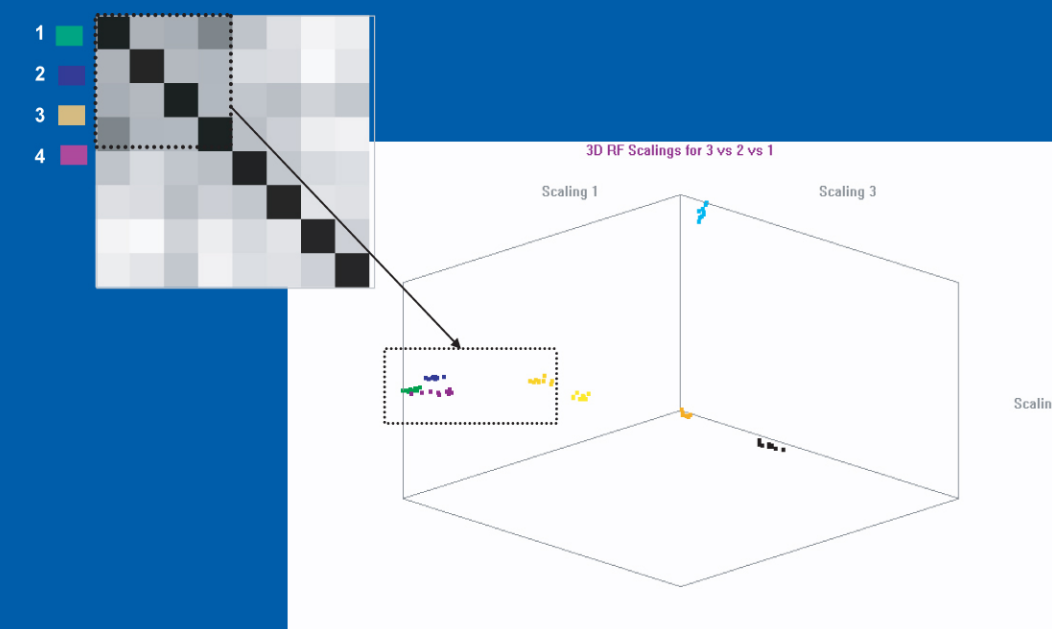| STANDARDS INPUT CLASSES	Class #	2CP-C (Fumarate) 1	2CP-C (Nitrate) 2	2CP-C (2-CP) 3	2CP-3 4	2CP-1 5	R 6	DS str BB1 7	Geo str SZ 8	Total # Spectra
2CP-C (Fumarate)	1									9
2CP-C (Nitrate)	2									9
2CP-C (2-CP)	3									9
2CP-3	4									9
2CP-1	5									9
R	6									9
DS str BB1	7									9
Geo str SZ	8									9

Scale: 0% — 100% (10% steps)

Classification with the Random Forest Algorithm: Nine processed spectra from each class were grouped successfully, manifested by the dark areas of this grid view. The darkness of areas in the grid is directly proportional to the strength of the correlation between each spectrum and the group into which it is classified by Random Forest. The grey areas represent similarity between different classes.

Class #	Class Name	Class #	Class Name
1	2CP-C {Fumarate}	5	2CP-1
2	2CP-C {Nitrate}	6	R
3	2CP-C {2-Chlorophenol}	7	DS str. BB1
4	2CP-3	8	Geo str. SZ

Alternative Representation of Random Forest Classification Results: 3-Dimensional view of the grouping of spectra, and association to the grid representation. The groups with greater similarity are found closer together in space in the figure. Each dot represents one spectra (9 spectra in each of 8 classes; one color for each class).

Conclusions

MALDI-TOF MS offers a rapid and simple alternative for fingerprint analysis of environmentally relevant microorganisms.

Mass spectral profiles can discriminate microorganisms by group and strain, providing the specificity required for environmental monitoring.

Spectra may offer information on the metabolic status of a particular strain at the time of sampling.

Future work will focus on:

- Identification of proteins; linking proteomics and genomics approaches
- Analysis of consortia and environmental samples